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L. O. HOWARD, Entomologist and Chief of Bureau.

THE CAUSE OF EUROPEAN FOUL BROOD.

BY

G. F. WHITE, M. D., PH. D.,
Expert in Bacteriology.

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HISTORICAL.

The purpose of this preliminary paper is to discuss briefly the exciting cause of European foul brood.

With diseased material furnished by Cheshire, Cheyne made a bacteriological study of foul brood. The latter isolated and described a bacterium from the brood dead of the disease and identified it as *Bacillus alvei*. Cheshire agreed that the identification was correct. A joint paper by these men appeared in 1885, and for about a decade and a half thereafter *Bacillus alvei* was generally considered to be the cause of foul brood. The disease which, it is believed, Cheyne studied is the one that has received the designation European foul brood.

This disease is also believed to be the one which William R. Howard worked upon and named "black brood." In 1900 he described as its cause a bacterium to which he gave the name *Bacillus milii*.

It is probable that Burri in 1906 was studying the same disease in Switzerland when he referred to "sour brood." In the disease he discovered a bacterium to which he referred as the "*guntheri*-forms."

Maassen in 1907, working probably with the same disease, apparently encountered the "*guntheri*-forms" reported by Burri and named the new species *Streptococcus apis*.

SOME EARLIER WORK BY THE WRITER ON EUROPEAN FOUL BROOD.

In 1907 the writer observed in European foul brood the bacterium which had been named *Streptococcus apis*. It was observed at the same time that there was present also another microorganism quite

similar in appearance, but clearly different from the one which Burri had observed and Maassen had named. All attempts, however, to cultivate this new species were unsuccessful. Until more was known about this organism it was referred to in 1908 as "*Bacillus Y*."

In 1907 the writer demonstrated that American foul brood, an infectious brood disease, could be produced by feeding to healthy colonies pure cultures of *Bacillus larvæ*. This fact emphasized the probability that if European foul brood is also caused by a bacterium this disease, too, could be produced by feeding pure cultures of the bacterium causing it.

To demonstrate this, it was desirable in the first place to determine whether or not the virus of European foul brood was present and active in the diseased brood. Healthy bees were fed sirup which contained a suspension of the diseased brood, and European foul brood was produced. This showed that the diseased brood did contain the virus and that the disease could be produced by feeding. This being done, pure cultures of *Bacillus alvei* isolated from the diseased material were substituted for the diseased brood in the inoculation experiment. Pure cultures of *Streptococcus apis* were isolated and used likewise. Then cultures of *Bacillus alvei* and cultures of *Streptococcus apis* were used simultaneously in making the inoculations. European foul brood was not produced in any of the experiments where pure cultures of either or both organisms were used. These facts were sufficient to eliminate tentatively *Bacillus alvei* and *Streptococcus apis* from the list of possible causes of European foul brood, and to justify a strong suspicion that the microorganism which was referred to as "*Bacillus Y*" bore a causal relation to the disease. It was necessary, however, to reckon with other factors before a more definite statement could be made.

A continuation of the work on the cause of European foul brood has yielded some interesting results. These will be briefly considered in this preliminary paper.

It has been observed in the examination of diseased brood that *Bacillus alvei* is frequently either absent, or present only in small numbers, in many of the larvæ which seem from gross appearance to be dead of European foul brood. Such samples have been received as a rule from localities in which apparently the disease had only recently appeared. Frequently, also, *Streptococcus apis* seemed to be absent, or present only in small numbers, in many of the larvæ which from gross appearance gave strong evidence of European foul brood. These bacteriological findings further strengthened the theory that neither *Bacillus alvei* nor *Streptococcus apis* is the primary exciting cause of European foul brood. Other inoculation experiments were performed, using pure cultures of these two species.

The results were always negative, confirming further similar results that had been previously obtained.

RECENT WORK BY THE WRITER.

While these facts were in a measure satisfactory, as tending strongly to indicate certain conclusions, there was still wanting that degree of conclusiveness which is always desired. By experimental inoculation and by the study of the brood sick or dead of the disease which was artificially produced, however, considerable information of the character hoped for has been obtained. The details of the technique used in making the inoculation will not be given in this brief report.

EXPERIMENTAL INOCULATIONS.

Diseased material from various localities was used for these inoculations. Some colonies were fed diseased brood that contained, as revealed by cultural examinations, large quantities of *Bacillus alvei;* other colonies were fed diseased material containing large numbers of *Streptococcus apis* as shown by cultures, and still others were fed diseased material which was demonstrated to contain neither *Bacillus alvei* nor *Streptococcus apis.* It was found that at the first appearance of the disease in each class of experiments the symptoms manifested by the sick larvæ were the same. Larvæ showing these early symptoms were studied bacteriologically. The examinations showed that whether or not the diseased material fed to the bees contained *Bacillus alvei* or *Streptococcus apis* these species were in the early stages of the disease either absent, or present in small numbers only. It is quite evident that the disease was not produced by species of bacteria which were absent at this early stage of the disease.

Continuing the bacteriological study of the larvæ in the early stages of the disease, some new species were found to be present. One bacterium especially is frequently encountered. This species is a small, slender rod, apparently nonmotile and nonspore bearing. It is to be known by the name *Bacterium eurydice.* Its description will appear in a later publication. Experimental colonies have been fed pure cultures of this species, but no disease has been produced. Tentatively, therefore, this species is not to be regarded as the cause of European foul brood.

Two other species of bacteria might be mentioned here as being of interest in connection with the study of the brood diseases. The first to be mentioned is a motile, spore-bearing, easily cultivatable rod. It is to receive the name *Bacillus orpheus.* It also will be described later. This species is occasionally found in very large num-

bers in samples of European foul brood. Feeding it in pure cultures has so far given negative results. This organism, then, can also be eliminated tentatively from the list of possible causes of this disease. The other species. mentioned by Lambotte in 1902 as *Bacillus mesentericus vulgaris*, may be said to belong to a group of bacteria found quite widely distributed in the apiary. Its infrequency in diseased brood and its occurrence in small numbers readily eliminates this species from the list of possible causes. Most of the bacteria that are met with in the study of European foul brood were therefore excluded tentatively from the list of possible causes of the disease.

The possibility of an ultramicroscopic virus was also considered. Brood sick or dead of European foul brood were removed from the combs and crushed. An aqueous suspension of this diseased material was then made in boiled water and filtered with the Berkefeld filter. The filtrate remained clear when incubated at different temperatures and cultures made from it produced no growth. Separate filtrations have been made of diseased brood received from various localities, but in no instance where healthy colonies were fed filtrate obtained in this way was European foul brood produced. The results of the experiments therefore justify the tentative conclusion that there is no filterable virus in European foul brood capable of producing the disease. To this extent, then, has the possibility of an ultramicroscopic virus been eliminated.

Having thus tentatively eliminated all the microscopically visible organisms except *Bacillus Y* from the list of possible causes and likewise eleminated the probability of an ultramicroscopic virus, the tentative conclusion was naturally reached that this remaining microorganism probably plays an important rôle in the etiology of European foul brood. Such a conclusion was all the more imperative since this organism had been encountered so frequently in the brood of this disease and since, moreover, there had been no other factor observed to which the exciting cause could be attributed.

SYMPTOMS MANIFESTED BY LARVÆ SICK OF EUROPEAN FOUL BROOD.

This conclusion led to a more extended study of this microorganism in the disease produced experimentally. The presence of disease can usually be detected in the experimental colony during the week that the feeding is begun. The first indication of it may be that only a portion of a larva is seen in a cell (fig. 1), the remaining portion having been removed by the bees. Aside from an observation of this kind the earliest indication one gets from the macroscopic (gross) examination is that sick larvæ are found among the uncapped brood. One should acquaint himself, therefore, with certain symptoms or signs manifested by sick larvæ during the course of the dis-

ease by which its presence can be diagnosed while the larvæ are still alive. Some of these will now be considered.

FIG. 1.—Larva sick of European foul brood, partly removed by the bees. (Original.)

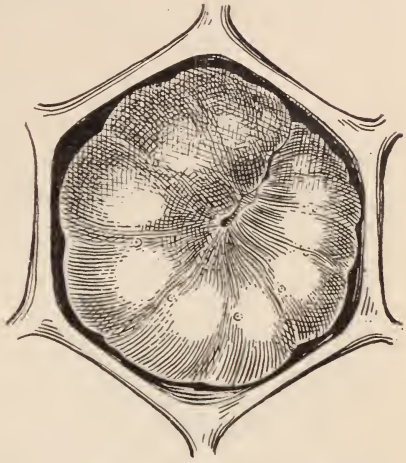

FIG. 2.—Healthy larva of the age represented in figure 4. (Original.)

The length of time that a developing bee is sick of European foul brood is variable. It can be stated in a general way that the three

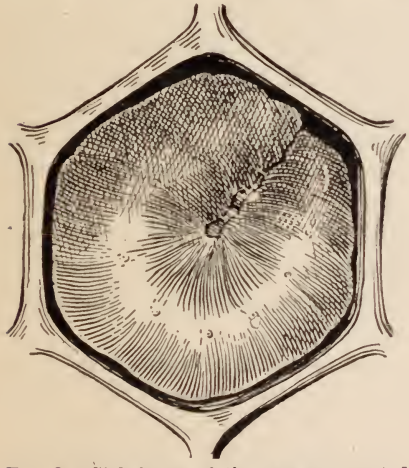

FIG. 3.—Sick larva of the age represented in figures 2 and 4. (Original.)

days just preceding the time when a larva would ordinarily be capped is the most favorable period for making a diagnosis from the gross examination alone.

When healthy larvæ of the age represented in figures 2, 3, 4, and 5 are slightly magnified a peristal-

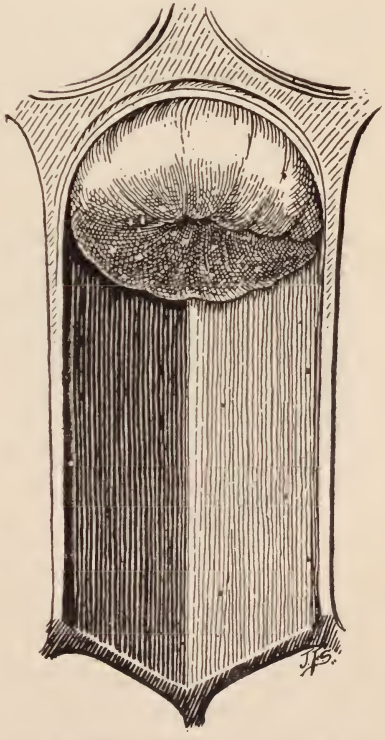

FIG. 4.—Sick larva with roof of cell removed. (Original.)

sislike motion of their bodies is easily seen, but larvæ of this same age when sick frequently exhibit a marked peristalsislike motion of their bodies which can be easily seen with the unaided eye. Some-

FIG. 5.—Sick larva which is more transparent than a healthy larva of the same age. (Original.)

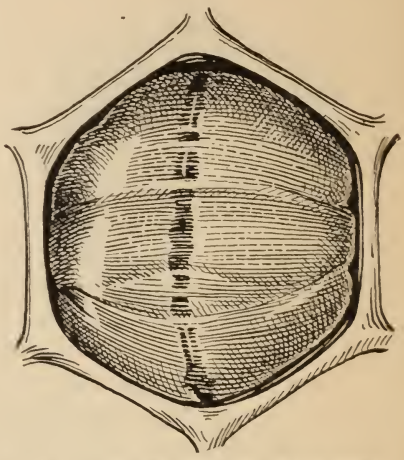

FIG. 6.—Healthy larva with dorsal wall turned toward the observer showing the narrow transparent area along the median dorsal wall. (Original.)

times the color of the larvæ assists in the selection of those that are diseased. If, instead of the glistening white or bluish-white appearance of healthy larvæ, one observes some that are more transparent (fig. 5), or that possess a very slight yellowish tint, frequently such larvæ are diseased. In the absence of the exaggerated peristalsislike movement, however, other tests should be applied, as the color symptom is at times deceptive.

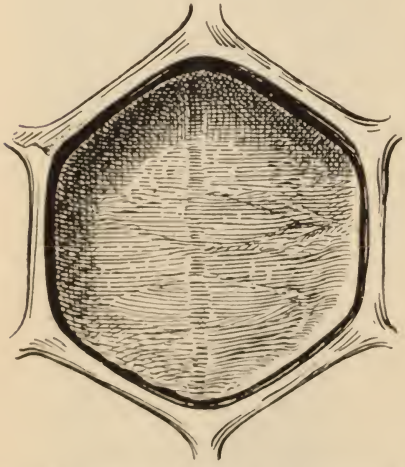

FIG. 7.—Sick larva of the age represented in figure 6. (Original.)

Figures 6 and 7 represent older larvæ than the preceding. These have turned themselves in the cell so as to present a dorsal portion to the observer. The narrow and quite transparent area frequently seen along the dorsal median line of a larva serves often a useful purpose in the diagnosis of European foul brood. In a healthy larva (fig. 6) a pollen-colored intestinal mass is frequently plainly visible through this transparent dorsal area. Microscopically this mass is easily demonstrated to be largely pollen. If, however, upon

inspection this intestinal mass appears white or yellowish white, the presence of European foul brood is almost certain. A modification of this simple inspection method may often be profitably used. This consists simply in turning the larva in the cell with a pair of forceps until the median dorsal line is exposed to the observer.

More frequently still, it will be found advantageous to remove the larva from the cell with the forceps. With a little care this can be done, leaving the larva intact. If the larva is diseased and the disease is sufficiently advanced, a whitish intestinal content can very often be plainly observed. In response to the muscular action of the larva this mass is frequently seen to be moved to and fro.

A POSITIVE TEST FOR THE DISEASE IN LIVING LARVÆ.

There is a sign represented in figure 8 which, in the experience of the writer, has proven thus far to be a positive symptom of European foul brood. When the age and condition of the diseased larva are favorable—and these frequently are—the sign can be quite easily and conveniently demonstrated in this way: Select a larva to be tested, approximately of the age represented by figures 2, 3, and 4; remove it from the cell and place it upon glass, preferably with a dark background; with a dissecting needle in each hand and with their points near together, pierce with both needles the wall of the larva near its head, avoiding the intestine; separate now the points of the needles so as to tear the body wall crosswise and continue to separate the two portions of the larva. If the larva is diseased and one is successful in applying the test, it will be found that the intestinal content will be stripped from and pulled out of the posterior and blind end (*b*, fig. 10) of the canal, obtaining results as represented in figure 8. In case of living, healthy larvæ the intestinal content can not be removed in this way.

This mass thus removed from the intestine, if examined microscopically, will be found, in general, to consist of a white or slightly yellowish-white mass along the longitudinal axis. This central mass is surrounded by a substance which is more or less transparent and mucuslike in appearance. The appearance of this outer portion, however, will vary in detail, depending in a great measure upon the stage of the disease when the examination is made.

The force which is applied in pulling the mass from the intestine frequently causes this enveloping substance to stretch and the inclosed whitish substance to break into segments as represented in *a* of figure 8. This is an earlier stage of the disease than that represented in either *b* or *c* of the same figure.

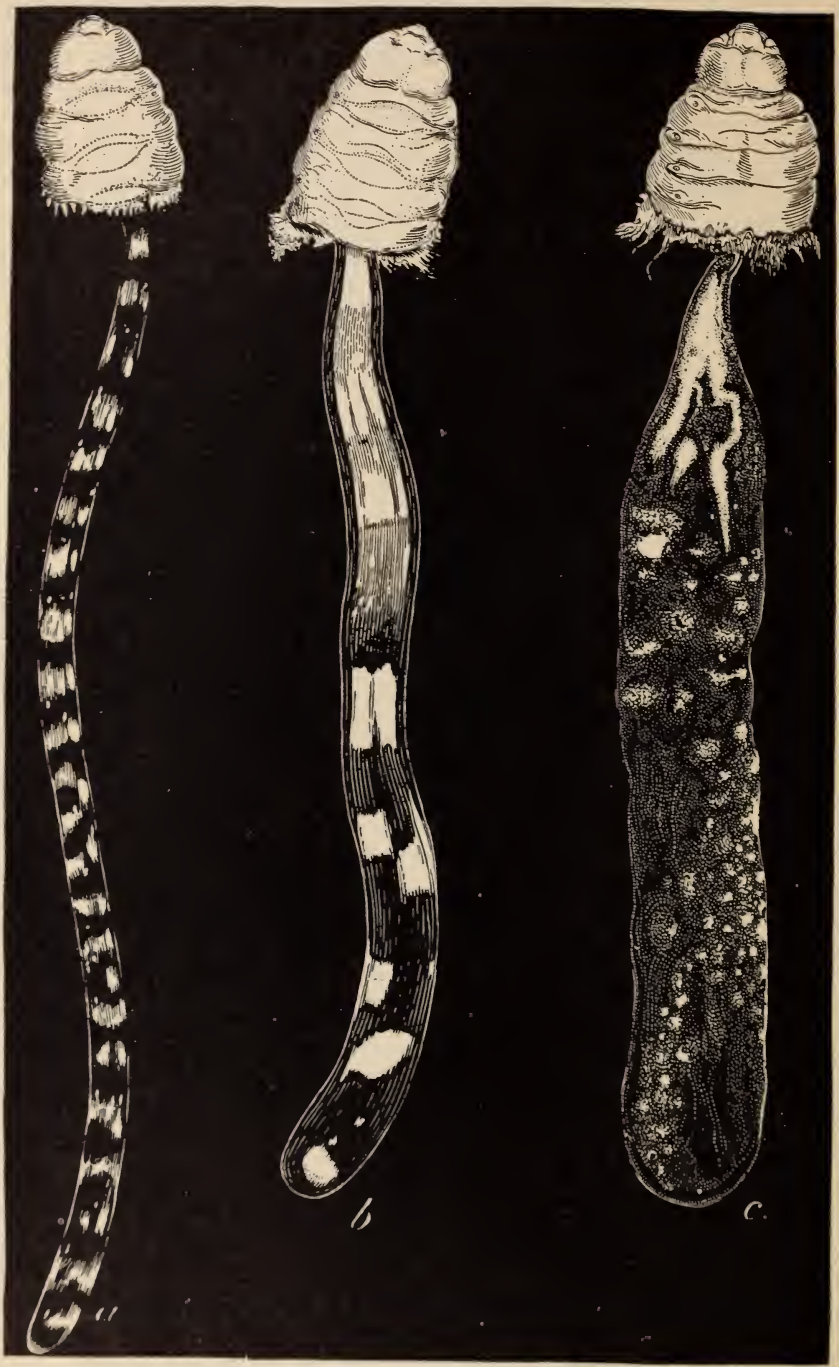

FIG. 8.—The intestinal content removed from larvæ sick of European foul brood but not yet dead of the disease. (Original.)

If the disease is more advanced than either stage represented in figure 8 when this test is applied, a portion of the intestinal content may flow out in the form of a sac, the wall of which is very easily broken. When broken the content of this saclike structure will flow out as a rather thin whitish or yellowish-white fluid containing small whitish granules that vary in size. If the disease is far advanced and the larva probably dead, the enveloping substance of the intestinal content is so easily broken that often only whitish or yellowish-white fluid with its granular content flows from the ruptured wall of the larva.

Figures 2 and 6 represent healthy larvæ, and at these ages the segments of the body are strongly marked off. Living larvæ at these ages, if suffering from European foul brood, frequently show these markings less distinctly as represented in figures 3, 4, and 7. This sign, too, may assist in the selection of larvæ that are suspected of being diseased.

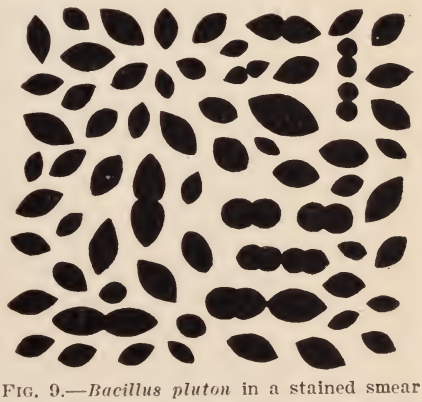

FIG. 9.—*Bacillus pluton* in a stained smear preparation from sick larvæ at stage represented in *a*, figure 8. (Original.)

THE VALUE OF EARLY SYMPTOMS IN THE DIAGNOSIS OF EUROPEAN FOUL BROOD.

These symptoms of European foul brood are some of the more important ones that are observed in sick larvæ or in those only recently dead. They are especially valuable in the study of the disease in the experimental colony. They have not been used by the apiarist for making a diagnosis. The symptoms of European foul brood that have been looked for by the bee keeper for the most part are the evidences of disease which obtain as a result of the death of the brood. The post-mortem symptoms as manifested by the dead larvæ themselves have been the most positive evidences used by the bee keeper in diagnosing the disease. It is hoped, however, that when they are well learned, the symptoms of European foul brood observed in living larvæ and in those very recently dead may prove of value in the apiary as well as in the experimental colony.

Practically all the later symptoms of European foul brood have also been observed during the course of the disease in the experimental colony. This fact is used as evidence that the disease which was produced in the experimental colony was the same as that encountered in the apiary. Since the diseased material for making the inoculations has been received from various sources and the disease produced was apparently the same in every case, the conclusion that

there is but one disease present in the condition which is being called European foul brood is, therefore, still further confirmed.

MICROSCOPIC STUDIES OF DISEASED LARVÆ.

Returning now to the discussion of European foul brood in the earlier stages, it should be emphasized that by a macroscopic examination alone it is not always possible to make a positive diagnosis of the presence or absence of disease in a larva. During the very earliest period of infection it is impossible from the gross examination alone to make a positive diagnosis of the presence of disease. Such is to be expected. About the time the larva dies there is a period at which one can not always be sure that the disease is present from a macroscopic examination alone. Between these stages there is a period in the course of the disease in the larvæ during which it is usually possible to make a diagnosis positive from the gross examination. Since a macroscopic examination alone is not always sufficient for making a positive diagnosis, one looks naturally to a microscopic examination for assistance.

During the course of the disease in the experimental colony the microscopic picture presented in the examination of diseased larvæ changes markedly. To begin the microscopic study, it is well to obtain the intestinal content as represented in *a*, figure 8. If a thin smear is made of the white growth-mass of this content and stained, it is found to consist almost entirely of forms represented in figure 9.

This organism is the one that the writer referred to in an earlier paper as "*Bacillus Y*." All attempts to cultivate this new species on artificial media have thus far been unsuccessful. Since considerable information has now been obtained concerning this organism the specific name "*pluton*" is now substituted for the "*Y*" in the term "*Bacillus Y*" and the species will now be known as *Bacillus pluton*. This organism is an unusual one and the classification has not yet been definitely determined. The generic term "Bacillus," therefore, may, and probably will, be changed later.

At the stage of the disease represented in *a*, figure 8, the majority of the individuals of this new species in general appear in stained preparations to be pointed at the ends (fig. 9). Some show both ends rather sharply pointed, others show only one end so pointed, the other end being rounded, while still others show both ends rounded. The individuals having this general form vary much in size. They are as a rule 1 μ or less in length, the breadth being about one-half the length. Forms in pairs frequently occur in a smear preparation made at this stage of the disease. These paired forms vary markedly in size and shape. (See fig. 9.) Accompany-

ing *Bacillus pluton*, *Bacterium eurydice* is frequently found at this stage of the disease, but in comparatively small numbers.

If the intestinal content in a later stage of the disease—for example, that represented in *b*, figure 8—is examined microscopically, *Bacillus pluton* is still found in very large numbers, and *Bacterium eurydice* when present will be relatively very much increased in numbers. A similar examination of the intestinal content represented in *c*, figure 8, will usually show *Bacillus pluton* in large numbers, *Bacterium eurydice* in increased numbers, and in addition one may find *Bacillus alvei* in comparatively small numbers.

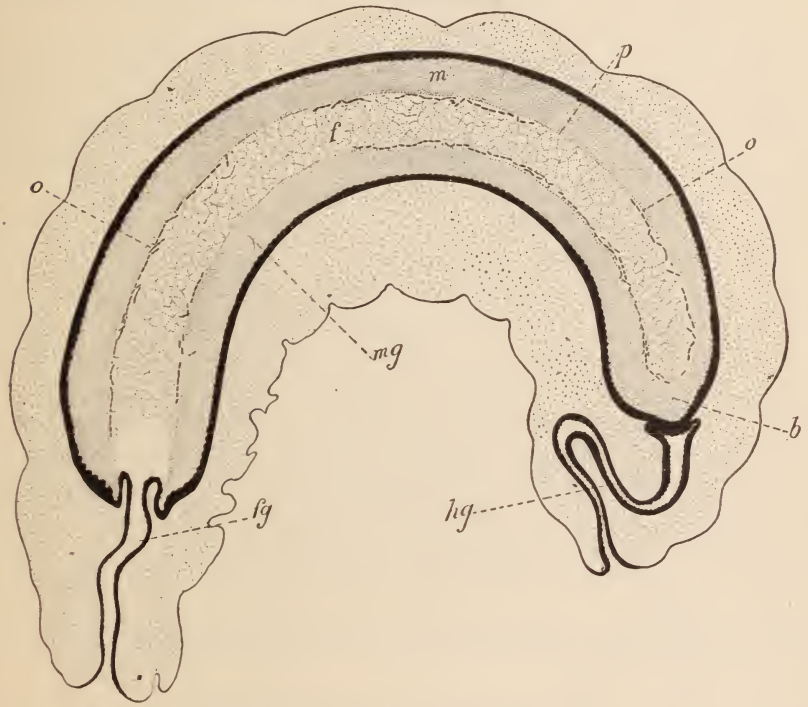

FIG. 10.—Schematic drawing representing a longitudinal section of a larva at an early stage of infection. The position of the invading organism, *Bacillus pluton*, is along and near the peritrophic membrane. (Original.)

By examining the fluid mass which flows from the body of a larva when the disease is far advanced and the body wall is broken, one usually finds, together with *Bacillus pluton*, bacteria of different species in considerable numbers.

From this point on in the decay of the larvæ the relative proportion of the different microorganisms present varies markedly. When *Bacillus alvei* is present it increases very rapidly in proportion to the others. This rapid increase of *Bacillus alvei* in the larvæ after the death of the larvæ accounts in a large measure for the frequency with which this species is mentioned in reports on this disease.

In living larvæ, therefore, in which European foul brood can be diagnosed from gross examination, it is found that bacteria usually accompany *Bacillus pluton*. This fact made desirable the study of the diseased larvæ in still earlier stages of the infection, i. e., during the period of incubation. This was done culturally in part, but principally by fixing and sectioning the younger larvæ from strongly infected experimental colonies. From such sections it was observed that *Bacillus pluton* was the first invader of the healthy larvæ.

Figure 10 represents schematically the condition in the larvæ at an early stage of infection. In this figure *fg* represents the foregut; *mg* the midgut, and *hg* the hindgut. At this age of the larvæ the posterior end of the midgut is closed, as represented at *b*. In the same figure, *m* represents that portion of the intestinal content lying in contact with the wall of the intestine; *f*, the central portion of the drawing, represents the food taken at this age; and *p* represents what seems to be a peritrophic membrane between the enveloping substance, *m*, and the paplike food substance, *f*, of the midgut.

In the growth of *Bacillus pluton* this parasite very early takes a position along the peritrophic membrane *p*, and just central to it (fig. 10). At this early stage of its growth this microorganism presents in general an appearance of being rod shaped with a strong tendency to grow in chains. As the disease advances and the growth-mass of this organism increases, the central portion of the lumen of the intestine becomes filled by a solid growth which is made up very largely of *Bacillus pluton*. During this stage of the disease the content can be removed from the posterior blind end of the midgut, as shown in figure 8. The relation of the central growth-mass to the surrounding mucuslike-appearing mass represented in *a*, *b*, and *c* of figure 8 is well demonstrated microscopically by sectioning these intestinal masses.

From the studies made thus far it would seem that *Bacillus pluton* is easily killed by heat.

PROBABLE EXPLANATIONS OF ERRORS AS TO THE EXCITING CAUSE OF EUROPEAN FOUL BROOD.

It is quite probable that others at different times have observed this new species, *Bacillus pluton*, but have failed to differentiate it from bacteria which were present and which appeared in the cultures made, leading them thus to erroneous statements concerning the disease and its exciting cause. For example, William R. Howard may have seen this organism microscopically in his so-called "black brood," but failed to differentiate it from some bacterium—*Bacillus milii* or *Bacillus alvei*—which he cultivated on artificial media. Burri may have seen it in the so-called "sour brood" and mistaken it

for the "*guntheri*-forms" which he observed in his cultures. Maassen mentions some difficulty experienced at times in obtaining *Streptococcus apis* from brood which on microscopic examination seemed to contain this bacterium. To explain this difficulty, he advanced the supposition that the Streptococcus was probably killed by acid produced by itself. The difficulty probably could be as well explained by supposing that Maassen failed to differentiate this parasite from the bacterium which he cultivated and described as *Streptococcus apis*.

IS THERE MORE THAN ONE DISEASE IN THE CONDITION KNOWN AS EUROPEAN FOUL BROOD?

The question now arises whether or not there is more than one disease in the condition now known as European foul brood. In Switzerland and in Germany there has been a tendency to diagnose the diseased brood in which *Bacillus alvei* is found as the foul brood of Cheshire and Cheyne and the diseased brood in which *Streptococcus apis* is found as "sour brood." From the facts at hand the writer is strongly inclined to believe that these two conditions are only the one disease, known in America as European foul brood. Enough evidence has not yet been obtained, however, to speak with complete positiveness on this point.

As secondary invaders some of the species of bacteria mentioned in this paper may and probably do exert an influence on the course of the disease in the larva and in the colony. To what extent these bacteria modify the disease is yet to be determined. Should it be found that *Bacillus alvei* actually causes an infectious brood disease, then such a disease should be called European foul brood, and the disease caused by *Bacillus pluton* would have to be differentiated from it.

Further details will not be given in this preliminary announcement but will be included in more technical papers which are being prepared.

SUMMARY AND CONCLUSIONS.

The steps taken in the writer's endeavor to find the cause of European foul brood may be briefly summarized as follows:

(1) *Bacillus alvei*, which has been so generally spoken of as the cause of foul brood, was isolated from diseased brood, and pure cultures of the organism in both the vegetative and spore forms were repeatedly fed to colonies of healthy bees with the result that foul brood was not produced in any instance. This fact cast a suspicion that *Bacillus alvei* was probably not the cause of a disease.

(2) By a study of many larvæ in samples of European foul brood it was frequently found that there were larvæ apparently dead of the disease that contained *Bacillus alvei* only in small numbers or not at

all. This increased the suspicion that *Bacillus alvei* was not the exciting cause of the disorder.

(3) In 1907 the writer proved that by feeding pure cultures of *Bacillus larvæ* to healthy bees American foul brood could be produced. This fact still further emphasized the doubt that was already entertained concerning the possibilities of *Bacillus alvei* in the etiology of European foul brood.

(4) By feeding diseased larvæ to healthy colonies it was found that European foul brood could be artificially produced, showing that this disease, too, could be produced by feeding, and that the virus was contained in the diseased brood.

(5) The sick larvæ of the disease thus artificially produced were frequently found, when examined, to be free from *Bacillus alvei*. This evidence, too, was damaging to the theory that *Bacillus alvei* is the cause of a brood disease.

(6) *Bacillus alvei* in this way was tentatively eliminated from the list of possible exciting causes of European foul brood. In a quite similar manner the other bacteria—*Streptococcus apis*, *Bacillus mesentericus vulgaris*, *Bacillus orpheus*, *and Bacterium eurydice*—were likewise eliminated from the list.

(7) Considerable quantities of filtrate from aqueous suspensions of crushed diseased larvæ were fed to healthy colonies and in no instance was European foul brood produced. This eliminated tentatively the probability of there being an ultramicroscopic virus in European foul brood capable of producing the disease.

(8) *Bacillus pluton*, therefore, was the only factor that was not so eliminated from the list of possible exciting causes of the disease and became thus the probable exciting cause of European foul brood.

(9) When this organism was studied in larvæ in which the disease could be suspected by inspection alone, one or more species of bacteria were sometimes found to be present also. These, when present, however, occurred in relatively small numbers.

(10) The disease was then studied in a still earlier stage; i. e., before its presence could be detected by gross examination of the larvæ. This was done by cultures in part, but principally by fixing and sectioning larvæ during the incubation period of the disease. This study demonstrated that in the production of the disease *Bacillus pluton* was the first invader of the healthy larvæ.

It will be noticed, therefore, that in the determination of the primary exciting cause of European foul brood two objects were accomplished: (1) All the factors in the list of possible exciting causes of the disease were eliminated except the one organism *Bacillus pluton*, and (2) by the study of infected larvæ soon after the infection took place, this parasite was found to be the first invader.

As a conclusion, it is the belief of the writer that sufficient evidence has now been obtained to justify the statement that *Bacillus pluton* is the primary exciting cause of a brood disease. This brood disease is now generally known in America as European foul brood. This opinion is rendered in accordance with views now generally accepted relative to the etiology of animal diseases.

There are, then, three principal brood diseases. Two of these—American foul brood, caused by *Bacillus larvæ*, and European foul brood, caused by *Bacillus pluton*—are known to be infectious. From these two diseases there must be differentiated the third one, an apparently noninfectious disorder, the so-called "pickled brood." Larvæ dead of this latter disease are practically free from micro-organisms. The exciting cause of this disorder is not yet known.

Approved:

JAMES WILSON,
Secretary of Agriculture.

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